Shenandoah and James River Basin Fish Kills: 2007 Passive Sampler Results Summary

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DEQ
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SHENANDOAH AND JAMES RIVER BASIN FISH KILLS: 2007 PASSIVE SAMPLER RESULTS SUMMARY

1.1. EXECUTIVE SUMMARY

In the spring of 2007, passive samplers were deployed at 12 locations in the Shenandoah and James River basins as part of fish kill investigations in these rivers. Passive sampler extracts were analyzed for 199 compounds including polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), pesticides, waste-indicator chemicals, pharmaceuticals, and hormones. Results from those analyses were originally reported in Alvarez *et al.* (2008a) and Alvarez *et al.* (2008b). This report summarizes those results in comparison to control site values, water quality standards, minimum published lethal effect levels, minimum published benchmark screening criteria, and statewide probabilistic monitoring data, when available.

Of 199 compounds analyzed, only 84 compounds exceeded control site values at one or more fish kill sites. Of those, only 6 (all agricultural pesticides) exceeded control site values at a majority of fish kill sites. These 6 were all agricultural pesticides, including, metolachlor, atrazine, simazine, prometon, chlorpyrifos, and p,p'-methoxychlor. None of these compounds exceeded control values at all fish kill sites.

Virginia water quality standards were only available for a few analyzed compounds, but none exceeded available standards. Statewide probabilistic monitoring data were available for PAHs, PCBs, and organochlorine pesticides. Several compounds (4 PAHs, total PCBs, and 9 organochlorine pesticides) exceeded the 90^{th} percentile of the statewide probabilistic data at one or more fish kill locations. This is not surprising, considering fish kill sites contain more urban and agricultural influences than a spatially random sampling of streams across the state.

To evaluate measured contaminant concentrations in relation to toxicological endpoints, measured concentrations were compared against minimum published lethal effect levels and minimum published benchmark screening criteria. Contaminant concentrations measured at fish kill sites were orders of magnitude below levels reported to produce lethal effects. PAHs were

more than 4000 times below minimum lethal effect levels, organochlorine pesticides were more than 500 times below minimum lethal effect levels, agricultural pesticides were more than 10,000 times below minimum lethal effect levels, and hormones were more than 12 times below minimum lethal effect levels. In addition, none of the compounds exceeded more conservative benchmark screening criteria.

In summary, passive sampler data collected from the Shenandoah and James River basins in 2007 produced no evidence that fish kills are a direct result of chemical contamination in the water column. This finding does not preclude the possibility of effects from chemicals not analyzed in this study or indirect effects from chemical contamination that could decrease overall fish health or increase susceptibility to disease. For instance, fish kill researchers have proposed that chemical contamination could cause immune suppression that leads to bacterial infection and death. This study reviewed the immunosuppression literature and did not find examples of immune suppression effects at contaminant levels measured in passive samplers from fish kill sites, however, the immunosuppression literature is very sparse and research is continuing in this area. Fish kill researchers are continuing to investigate the role of bacterial infection and potential immune suppression effects.

1.2. BACKGROUND

Over the past several years, recurring fish kills have been observed in the Shenandoah River and Upper James River basins. These fish kills have been widespread and have exhibited distinct characteristics that led researchers to assume that the kills are linked and caused by a common stressor or set of stressors. These fish kills have occurred in the spring, beginning in April as water temperatures quickly warm, and subsiding in June as warmer temperatures stabilize. During this 2-3 month period, dead and dying fish have been commonly observed throughout long stretches of affected rivers with no clear upstream or downstream boundaries. The fish kills have primarily affected adult smallmouth bass (*Micropterus dolomieu*) and redbreast sunfish (*Lepomis auritus*). Other fish species and juveniles of the same species have been affected to a lesser degree or have not been affected. Lastly, mortality in the affected fish has often been accompanied or preceded by skin lesions.

These characteristic fish kills also have appeared to move from basin to basin. The first observation of this type of fish kill was reported in 2002 in the South Branch of the Potomac River in West Virginia. In 2004, the fish kills were first observed in Virginia in the North Fork of the Shenandoah River. The fish kills then moved to the South Fork of the Shenandoah River in 2005 and affected both forks and the main stem of the Shenandoah River in 2006. In 2007, the fish kills reoccurred in both forks of the Shenandoah River, and the kills began affecting the Upper James River and Cowpasture River. In the Cowpasture River, fish kills and lesioned fish were primarily reported in the lower reaches (mostly downstream of Route 39). Problems in the James River were most severe upstream of I-81 (near the Town of Buchanan), but also were seen as far downstream as Lynchburg.

In 2005, the Shenandoah River Fish Kill Task Force was formed to investigate the cause of the fish kills. This group is a collaborative partnership consisting of government, citizens, universities, and non-profit environmental organizations. The Virginia Department of Environmental Quality (DEQ) also formed a Research Advisory Committee (RAC) to guide the fish kill investigations and synthesize results from various researchers. One hypothesis that the Task Force and the RAC was interested in investigating was that chemical pollutants present in the water during the spring were responsible for causing the fish kills through direct toxicity or indirectly through suppressing immune system response. To begin investigating this hypothesis, passive samplers were deployed in fish kill affected areas and control areas in the spring of 2007. Extracts from passive samplers were analyzed for nearly 200 polar and non-polar organic chemicals. This report describes the results and analysis of passive sampler data in relation to Shenandoah River and Upper James River fish kills.

1.3. METHODS

1.3.1. Passive Samplers

Two types of passive samplers were deployed at each site. Semipermeable membrane devices (SPMDs) were deployed to sequester hydrophobic organic chemicals, and polar organic chemical integrative samplers (POCIS) were deployed to sequester more hydrophilic polar organics. SPMDs were constructed of purified triolein within a semipermeable low-density polyethylene tubing, as described by Alvarez *et al*. (2008a) in Appendix A. POCIS were

constructed of a chemical sequestration medium enclosed between two polyethersulfone membranes, as described by Alvarez *et al*. (2008a) in Appendix A Both SPMDs and POCIS were deployed in protective canisters as shown in Figure 1.

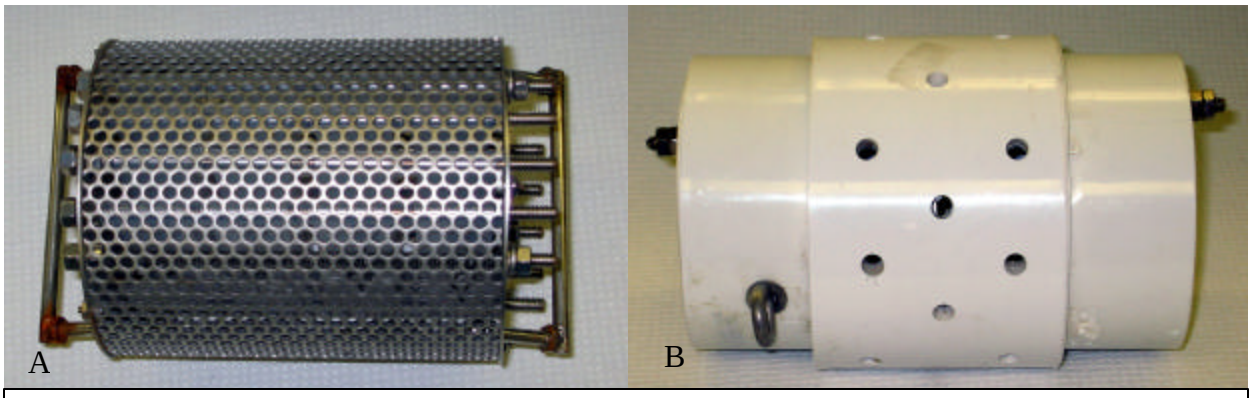

Figure 1. SPMD (A) and POCIS (B) Devices Deployed in Fish Kill Investigations.

1.3.2. Sampling Sites

Passive samplers were deployed at 12 sampling locations in the Shenandoah River and Upper James River basins (Table 1 and Figure 2). The Friends of the North Fork of the Shenandoah River (the Friends) deployed passive samplers at NF-Mt. Jackson and NF-Woodstock. The remaining passive samplers were deployed by DEQ. DEQ deployed samplers for 42 days from late March to early May. The Friends deployed two sequential sets of passive samplers. The first was deployed slightly earlier than the DEQ set (3/10/07 – 4/29/07) and for a slightly longer time period (50 days). The second set was deployed later in the spring, from the end of April to early June.

At the time of study planning, the Cowpasture R., Maury R., and Cedar C. sites were selected to represent control sites unaffected by fish kills. However, during and following deployment, extensive fish kills were observed in the Cowpasture R. For the analysis presented in this report, only the Maury R. and Cedar C. sites were treated as controls for comparison with fish kill sites.

1.3.3. Sample Analysis

Passive samplers were extracted and analyzed by the U.S. Geological Survey (USGS) as described by Alvarez *et al.* (2008a, 2008b) in Appendix A and B. Samples from NF-Mt. Jackson and NF-Woodstock, which were deployed by the Friends, were analyzed and results reported

separately (Appendix B) from samples deployed by DEQ (Appendix A). Samples deployed by the Friends included field blank samples, while samples deployed by DEQ did not employ field blanks. This resulted in some inconsistencies in the data sets from each source. Field blank results were subtracted from analytical results of samples collected by the Friends, while analytical results of DEQ-collected samples were simply reported as analyzed.

Passive sampler extracts were analyzed for 199 compounds representing the following classes: polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides and polychlorinated biphenyls (PCBs), agricultural pesticides, waste-indicator chemicals, pharmaceuticals, hormones, and a combined measure of estrogenic potential (Table 2). The full list of compounds analyzed from passive samplers is reported by Alvarez *et al.* (2008a) in Appendix A.

Table 1. Passive Sampler Deployment Locations.

River	Location	Site ID	Deployment Date		Duration (d)	Replicates
			From	To		
Shenandoah	Berryville	Shen-Berryville	3/28/2007	5/9/2007	42	1
NF Shenandoah	Cootes Store	NF-Cootes Store	3/22/2007	5/3/2007	42	1
	Mt. Jackson	NF-Mt. Jackson	3/10/2007	4/29/2007	50	2
			4/29/2007	6/9/2007	41	2
	Woodstock	NF-Woodstock	3/10/2007	4/29/2007	50	2
			4/29/2007	6/9/2007	41	2
	Strasburg	NF-Strasburg	3/22/2007	5/3/2007	42	1
Linville Creek	Broadway WWTP	Linville C.	3/22/2007	5/3/2007	42	1
SF Shenandoah	White House	SF-Whitehouse	3/23/2007	5/4/2007	42	1
North River	Port Republic	North R.	3/22/2007	5/3/2007	42	1
South River	Harriston	South R.	3/22/2007	5/3/2007	42	1
Cowpasture River	Walton Tract	Cowpasture R.	3/23/2007	5/4/2007	42	1
Maury River	at Mill Creek	Maury R.	3/23/2007	5/4/2007	42	1
Cedar Creek	Stalnaker Property	Cedar C.	3/22/2007	5/3/2007	42	1
Total =	**12 sites**					

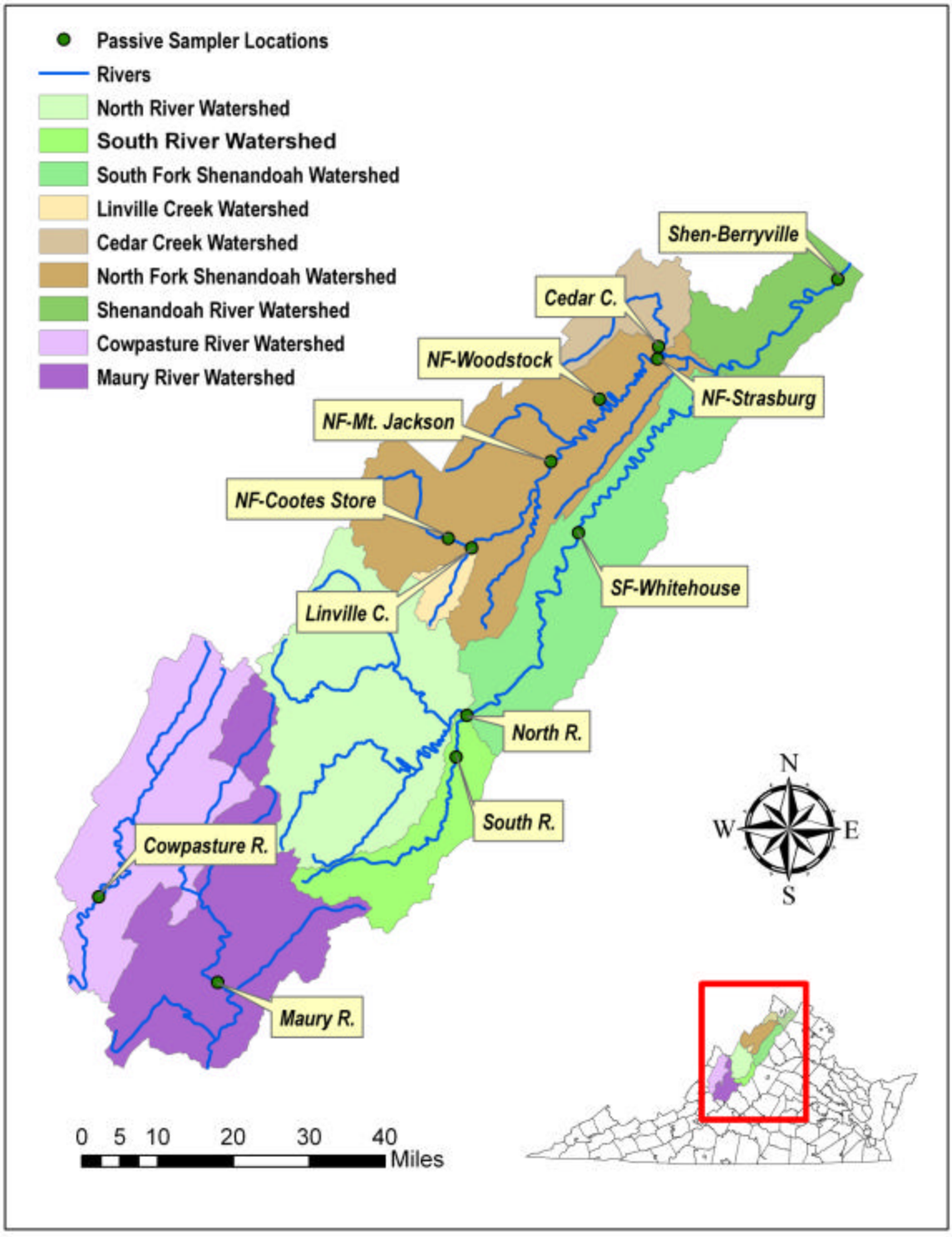

Figure 2. Map of Passive Sampler Deployment Locations for Fish Kill Investigations.

The USGS analyzed contaminant concentrations in passive sampler extracts and reported the result as either extract concentrations (e.g., ng/POCIS) or estimated water column concentrations (e.g., ng/L). In order to translate the measured extract concentrations to water column concentrations, information on SPMD and POCIS uptake rates is needed. For many compounds analyzed in this study, the uptake kinetics have been established, and instream water column concentrations were estimated. For other compounds, the kinetics have not yet been established, and results were only reported in units of mass per POCIS sample. For these compounds, comparisons can only be made between sites and not with effect level data. For compounds expressed as estimated water column concentrations, comparisons were made with control sites, water quality standards, minimum published lethal effect levels, minimum published benchmark screening criteria, and statewide probabilistic monitoring data.

Table 2. Classes of Compounds Analyzed from Passive Samplers.

Class of Compounds	# of Parameters	Sampler Type	Units
PAHs	34	SPMD	pg/L
Organochlorine Pesticides and PCBs	34	SPMD	pg/L
Agricultural Herbicides and Pesticides	35	POCIS	ng/L
Waste-indicator Chemicals	61	POCIS	ng/POCIS
Pharmaceuticals	30	POCIS	ng/POCIS
Hormones	4	POCIS	ng/L
Estrogenic Potential	1	POCIS	ng E2/POCIS
Total =	**199 Parameters**		

1.3.4. Results Analysis

Analytical results from DEQ-deployed passive samplers were reported by Alvarez *et al.* (2008a) in Appendix A, and analytical results from Friends-deployed passive samplers were reported by Alvarez *et al.* (2008b) in Appendix B. This report summarizes those analytical results in comparison to established effect levels or benchmark values from the literature to determine if any compound was detected at levels that would be responsible for causing the fish kills.

Results from fish kill sites were first compared to control site results. The Maury R. and Cedar C. sites were used as the control sites for this comparison. For each compound, the number of

fish kill sites exceeding control site values was recorded. Any compound responsible for causing fish kills would be expected to exceed control values at most, if not all fish kill sites.

Secondly, results were compared against Virginia Water Quality Standards, where available. Many of the compounds analyzed are "emerging contaminants" and states have not yet developed water quality standards for these compounds. For those that do have established standards, the freshwater chronic water quality criterion was compared to the maximum reported value from passive samplers at fish kill sites.

Results were also compared to effect levels cited in the scientific literature. Effect level data were extracted from the Environmental Protection Agency's (EPA) ECOTOX Database (USEPA, 2008a). ECOTOX is a searchable database for locating single chemical toxicity data for aquatic life, as well as terrestrial plants and wildlife. The database contains toxicity data derived predominantly from the peer-reviewed literature. Because numerous effect levels are reported for an individual compound in the database, the following screening procedures were used to identify the most representative effect level. If effect level data were available for the smallmouth bass genus (*Micropterus*) or sunfish genus (*Lepomis*), the minimum reported effect level for lethality or mortality endpoints was selected for comparison with passive sampler data. If effect level data were not available for either of these genera, then the minimum reported effect level for lethality or mortality endpoints from any fish species was selected. The lethality (or mortality) endpoint was selected for comparison, because it is the most prevalent and comparable endpoint in the database, and because mortality is the observed response at fish kill locations. Once the minimum reported lethal effect level was obtained, this value was compared to the maximum reported value from passive samplers at fish kill sites.

Results were also compared to benchmark screening criteria obtained from Oak Ridge National Laboratory's Risk Assessment Information System (ORNL, 2008). ORNL has developed an online tool to retrieve ecotoxicological benchmarks from a variety of sources. Benchmark screening criteria are used to identify chemical concentrations in environmental media that are at or below thresholds for effects to ecological receptors. For this study, 20 different sources of surface water benchmark screening criteria were evaluated for each compound (if available). These sources included EPA and Canadian criteria, as well as published criteria from the peer-

reviewed literature. For each compound, the minimum value of benchmark screening criteria was used for comparison with the maximum reported value from passive samplers at fish kill sites. These benchmark screening criteria are typically based upon sublethal effect level data for a wide variety of species and often contain additional safety factors, making them very conservative estimates of effect thresholds.

Lastly, passive sampler results were compared with probabilistic monitoring data previously collected by DEQ. In 2003, DEQ deployed SPMDs at 41 probabilistically selected stations across the Commonwealth (Cranor *et al.*, 2005). The probabilistic monitoring program is designed to achieve a random and representative sampling of waters across Virginia. The SPMDs deployed in 2003 were analyzed for a smaller subset of compounds than analyzed in this study, but for those compounds that overlapped, results were compared between the two studies. Passive sampler results from the current study were compared to the 90^{th} percentile of statewide probabilistic data to determine if results at fish kill sites were considerably higher than results elsewhere in the state.

1.4. RESULTS

1.4.1. PAHs

A total of 34 PAH compounds were analyzed from passive samplers. Of those 34 compounds, 6 were not detected at any site. Another 8 compounds were higher at control sites than at any of the fish kill locations. The remaining 20 compounds exceeded control values at one or more fish kill sites (Table 3). Measured values at individual sites exceeded control values by 1.2 to 26 times, but none of the compounds exceeded control values at more than 3 of the 10 fish kill sites. NF-Strasburg was the most common station exceeding control values, exhibiting the highest measured values for 14 of the 20 compounds.

Table 3. Comparison of PAH Levels at Fish Kill Sites with Control Sites.

Compound	# Sites Exceeding Control Values	Maximum Measured Value (pg/L)	Location of Maximum Value	Ratio of Maximum Concentration to Control Value
Benzo[b]fluoranthene	2	200	NF-Strasburg	26.0
2,3,5-trimethylnaphthalene	2	220	SF-Whitehouse	22.0
Benzo[a]pyrene	2	120	NF-Strasburg	13.2
Benzo[k]fluoranthene	2	110	NF-Strasburg	12.8
Benzo[g,h,i]perylene	1	130	NF-Cootes Store	10.8
3,6-dimethylphenanthrene	3	190	NF-Strasburg	4.6
1,2-dimethylnaphthalene	1	93	NF-Woodstock	4.4
1-ethylnaphthalene	1	72	NF-Woodstock	4.2
4-methylbiphenyl	2	590	Cowpasture R.	3.0
Benzo[e]pyrene	2	130	NF-Strasburg	2.6
Chrysene	2	670	NF-Strasburg	2.3
2-methylfluoranthene	2	94	NF-Strasburg	2.3
Benzo[b]naphtho[2,1-d]thiophene	2	94	NF-Strasburg	2.2
2-methylphenanthrene	3	410	NF-Strasburg	2.1
Perylene	2	260	NF-Cootes Store	2.0
Fluoranthene	2	2800	NF-Strasburg	1.8
Dibenzothiophene	2	140	NF-Strasburg	1.6
Phenanthrene	3	1900	NF-Strasburg	1.6
Pyrene	1	1200	NF-Strasburg	1.4
Benz[a]anthracene	1	100	NF-Strasburg	1.2
Naphthalene	0	Less Than Control Value At All Sites		
Acenaphthene	0			
Fluorene	0			
Anthracene	0			
2-methylnaphthalene	0			
1-methylnaphthalene	0			
Biphenyl	0			
1-methylfluorene	0			
Acenaphthylene	Not Detected At Any Site			
Indeno[1,2,3-c,d]pyrene				
Dibenz[a,h]anthracene				
Benzo[b]thiophene				
9-methylanthracene				
3-methylcholanthrene				

The 20 PAH compounds that exceeded control values at one or more sites were then evaluated against effect level data, benchmark screening criteria, and probabilistic data. Water quality standards were not available for any of the PAH compounds.

Table 4 compares the measured PAH values at fish kill sites with minimum effect levels published in the ECOTOX database. None of the PAHs measured at fish kill sites exceeded published lethal effect levels. With measured values in the pg/L range and typical effect levels for PAHs in the ug/L range, measured values were 3 to 7 orders of magnitude below relevant effect levels.

Effect level data were not available for all of the PAH compounds. Twelve compounds, primarily the methyl- and ethylated PAHs, could not be compared to effect levels. It is unlikely that any of these compounds would approach levels of effect, since the measured values range only from 72 to 590 pg/L and effect levels for these methyl- and ethylated PAHs are not likely to be 3 orders of magnitude lower than effect levels for the parent compounds.

Table 4. Comparison of Measured PAH Concentrations to Literature Cited Effect Levels.

Compound	Maximum Measured Value (pg/L)	Minimum Lethal Effect Level (ug/L)	Ratio of Effect Level to Measured Value
Fluoranthene	2800	12.3	4.4E+03
Benz[a]anthracene	100	1.8	1.8E+04
Pyrene	1200	25.6	2.1E+04
Benzo[a]pyrene	120	5.6	4.7E+04
Phenanthrene	1900	180	9.5E+04
Dibenzothiophene	140	700	5.0E+06
Chrysene	670	10000	1.5E+07
1,2-dimethylnaphthalene	93	1990	2.1E+07
Benzo[b]fluoranthene	200	Effect Level Data Not Available	
Benzo[k]fluoranthene	110		
Benzo[g,h,i]perylene	130		
1-ethylnaphthalene	72		
4-methylbiphenyl	590		
2,3,5-trimethylnaphthalene	220		
2-methylphenanthrene	410		
3,6-dimethylphenanthrene	190		
2-methylfluoranthene	94		
Benzo[b]naphtho[2,1-d]thiophene	94		
Benzo[e]pyrene	130		
Perylene	260		

Table 5 compares the measured PAH values at fish kill sites with minimum benchmark screening criteria. None of the PAH levels measured at fish kill sites exceeded published benchmark screening criteria. Measured values ranged from 1.4 orders of magnitude (14 times) to over 4

orders of magnitude below screening criteria. Fluoranthene and Pyrene were within 2 orders of magnitude of screening criteria levels and were the closest to approaching those levels. As was the case for effect level data, benchmark screening criteria were not available for many of the methyl- and ethylated PAHs.

Table 5. Comparison of Measured PAH Concentrations to Benchmark Screening Criteria.

Compound	Maximum Measured Value (pg/L)	Minimum Benchmark Screening Level (ug/L)	Ratio of Screening Level to Measured Value
Fluoranthene	2800	0.04	1.43E+01
Pyrene	1200	0.025	2.08E+01
Benzo[a]pyrene	120	0.014	1.17E+02
Benz[a]anthracene	100	0.018	1.80E+02
Phenanthrene	1900	0.4	2.11E+02
Chrysene	670	7	1.04E+04
Benzo[b]fluoranthene	200	9.07	4.54E+04
Benzo[g,h,i]perylene	130	7.64	5.88E+04
Benzo[k]fluoranthene	110	Benchmark Screening Data Not Available	
1-ethylnaphthalene	72		
1,2-dimethylnaphthalene	93		
4-methylbiphenyl	590		
2,3,5-trimethylnaphthalene	220		
Dibenzothiophene	140		
2-methylphenanthrene	410		
3,6-dimethylphenanthrene	190		
2-methylfluoranthene	94		
Benzo[b]naphtho[2,1-d]thiophene	94		
Benzo[e]pyrene	130		
Perylene	260		

Table 6 compares the measured PAH values at fish kill sites with 90th percentile statewide SMPD data. Four PAH compounds (fluoranthene, benzo(b)fluoranthene, benzo(a)pyrene, and pyrene) exceeded the statewide 90th percentile. This is not surprising considering the statewide probabilistic monitoring network contains many remote locations unimpacted by urban sources of PAHs and point source discharges that are prevalent within the fish kill watersheds.

Table 6. Comparison of Measured PAH Concentrations to Statewide 90th Percentile.

Compound	Maximum Measured Value (pg/L)	90th Percentile of Statewide Data (pg/L)	Ratio of Statewide 90th %tile Level to Measured Value
Fluoranthene	2800	1331	0.48
Benzo[b]fluoranthene	200	150	0.75
Benzo[a]pyrene	120	108	0.90
Pyrene	1200	1091	0.91
Benzo[k]fluoranthene	110	125	1.14
Phenanthrene	1900	2620	1.38
Benz[a]anthracene	100	145	1.45
Chrysene	670	1817	2.71
Benzo[g,h,i]perylene	130	401	3.08
1-ethylnaphthalene	72	Statewide Probabilistic Data Not Available	
1,2-dimethylnaphthalene	93		
4-methylbiphenyl	590		
2,3,5-trimethylnaphthalene	220		
Dibenzothiophene	140		
2-methylphenanthrene	410		
3,6-dimethylphenanthrene	190		
2-methylfluoranthene	94		
Benzo[b]naphtho[2,1-d]thiophene	94		
Benzo[e]pyrene	130		
Perylene	260		

In summary, several PAHs (fluoranthene, benzo(b)fluoranthene, benzo(a)pyrene, and pyrene) were higher at fish kill locations than is common across the state, however, PAH concentrations were well below published effect levels or benchmark screening criteria. Based on the measured concentrations of PAHs in passive samplers, it is unlikely that PAHs are a primary cause of fish kills in the Shenandoah and James River basins.

1.4.2. Organochlorine Pesticides and PCBs

A total of 33 organochlorine pesticides were analyzed from SPMD extracts. Total PCBs were also analyzed with this group of compounds, for a total of 34 compounds. Of those 34 compounds, only 3 were not detected at any site. Another 3 compounds were higher at control sites than at any of the fish kill locations. The remaining 28 compounds exceeded control values at one or more fish kill sites (Table 7). Measured values at individual sites exceeded control values by 1.0 to 45.2 times. Most compounds only exceeded control values at a few (3 or fewer)

fish kill sites, however, 2 compounds (chlorpyrifos and p,p'-methoxychlor) exceeded control values at a majority of fish kill sites (6 or 7 of the 10 fish kill sites). NF-Strasburg was the most common station exceeding control values, exhibiting the highest measured values for 13 of the 28 compounds.

Table 7. Comparison of Organochlorine Pesticide and PCB Levels at Fish Kill Sites with Control Sites.

Compound	# Sites Exceeding Control Values	Maximum Measured Value (pg/L)	Location of Maximum Value	Ratio of Maximum Concentration to Control Value
Trifluralin	3	19	NF-Mt. Jackson	45.2
delta-Benzenehexachloride (d-BHC)	3	75	NF-Woodstock	27.8
o,p'-DDD	5	29	NF-Strasburg	10.7
Dacthal	4	29	North R.	10.7
alpha-Benzenehexachloride (a-BHC)	2	47	North R.	10.0
Oxychlordane	5	7.1	North R.	8.7
Chlorpyrifos	7	550	NF-Mt. Jackson	7.5
beta-Benzenehexachloride (b-BHC)	3	110	North R.	5.2
p,p'-Methoxychlor	6	56	NF-Woodstock	4.7
Total PCBs	4	1600	NF-Strasburg	4.3
p,p'-DDD	1	79	NF-Strasburg	3.4
Hexachlorobenzene (HCB)	3	35	NF-Strasburg	3.2
cis-Nonachlor	3	18	NF-Strasburg	3.0
trans-Nonachlor	3	38	NF-Strasburg	2.5
trans-Permethrin	1	140	NF-Strasburg	2.5
cis-Chlordane	3	54	NF-Strasburg	2.5
p,p'-DDE	3	64	NF-Strasburg	2.3
Heptachlor Epoxide	3	42	SF-Whitehouse	2.1
o,p'-DDT	2	21	NF-Cootes Store	1.9
cis-Permethrin	3	120	NF-Woodstock	1.8
Endrin	3	36	NF-Mt. Jackson	1.8
Lindane	2	240	SF-Whitehouse	1.7
trans-Chlordane	2	37	NF-Strasburg	1.7
Dieldrin	4	56	NF-Strasburg	1.6
Pentachloroanisole (PCA)	2	59	NF-Strasburg	1.6
Heptachlor	1	1.2	NF-Woodstock	1.5
Endosulfan-II	1	510	NF-Strasburg	1.4
Endosulfan Sulfate	1	310	NF-Mt. Jackson	1.0
o,p'-DDE	0	Less Than Control Value At All Sites		
Endosulfan	0			
p,p'-DDT	0			
Tefluthrin	Not Detected At Any Site			
Diazinon				
Mirex				

Of the 28 organochlorine pesticide and PCB compounds, only 5 have established freshwater chronic water quality criteria in Virginia. These 5 are compared against the respective water quality standards in Table 8. All measured concentrations were well below Virginia water quality standards. Maximum measured values ranged from 75 to over 3000 times below the respective water quality standard.

Table 8. Comparison of Measured Organochlorine Pesticide and PCB Concentrations to Virginia Water Quality Standards.

Compound	Maximum Measured Value (pg/L)	Freshwater Chronic Water Quality Standard (ug/L)	Ratio of WQS Level to Measured Value
Chlorpyrifos	550	0.041	7.45E+01
Heptachlor Epoxide	42	0.0038	9.05E+01
Dieldrin	56	0.056	1.00E+03
Endrin	36	0.036	1.00E+03
Heptachlor	1.2	0.0038	3.17E+03
Trifluralin	19	No Water Quality Standard Available	
Hexachlorobenzene (HCB)	35		
alpha-Benzenehexachloride (a-BHC)	47		
Pentachloroanisole (PCA)	59		
Lindane	240		
beta-Benzenehexachloride (b-BHC)	110		
delta-Benzenehexachloride (d-BHC)	75		
Dacthal	29		
Oxychlordane	7.1		
trans-Chlordane	37		
trans-Nonachlor	38		
cis-Chlordane	54		
p,p'-DDE	64		
o,p'-DDD	29		
cis-Nonachlor	18		
o,p'-DDT	21		
p,p'-DDD	79		
Endosulfan-II	510		
Endosulfan Sulfate	310		
p,p'-Methoxychlor	56		
cis-Permethrin	120		
trans-Permethrin	140		
Total PCBs	1600		

Table 9 compares the measured organochlorine pesticide and PCB values at fish kill sites with minimum effect levels published in the ECOTOX database. None of the compounds measured at fish kill sites exceeded published lethal effect levels. With measured values in the pg/L range

and typical effect levels for organochlorine pesticides in the ug/L range, measured values were 2 to 10 orders of magnitude below relevant effect levels. Effect level data were available for all but 3 of the organochlorine pesticides (trans-nonachlor, cis-nonachlor, and o,p'-DDD). Trans- and cis-nonachlor are components of technical chlordane, and o,p'-DDD is a component of DDD, so effect level data for those parent technical mixtures would partially include the effects of these compounds.

Table 9. Comparison of Measured Organochlorine Pesticide and PCB Concentrations to Literature Cited Effect Levels.

Compound	Maximum Measured Value (pg/L)	Minimum Lethal Effect Level (ug/L)	Ratio of Effect Level to Measured Value
Chlorpyrifos	550	0.29	5.27E+02
Endosulfan-II	510	1	1.96E+03
Endrin	36	0.19	5.28E+03
Hexachlorobenzene (HCB)	35	0.31	8.86E+03
Endosulfan Sulfate	310	10	3.23E+04
Total PCBs	1600	54	3.38E+04
Dieldrin	56	2.8	5.00E+04
Lindane	240	12.5	5.21E+04
trans-Permethrin	140	14	1.00E+05
Heptachlor Epoxide	42	5.3	1.26E+05
cis-Chlordane	54	7.09	1.31E+05
cis-Permethrin	120	25	2.08E+05
p,p'-Methoxychlor	56	14	2.50E+05
Oxychlordane	7.1	2.45	3.45E+05
Trifluralin	19	8.4	4.42E+05
p,p'-DDD	79	42	5.32E+05
o,p'-DDT	21	25	1.19E+06
trans-Chlordane	37	50.5	1.36E+06
delta-Benzenehexachloride (d-BHC)	75	120	1.60E+06
alpha-Benzenehexachloride (a-BHC)	47	120	2.55E+06
p,p'-DDE	64	240	3.75E+06
Heptachlor	1.2	10	8.33E+06
beta-Benzenehexachloride (b-BHC)	110	1100	1.00E+07
Pentachloroanisole (PCA)	59	650	1.10E+07
Dacthal	29	700000	2.41E+10
trans-Nonachlor	38	Effect Level Data Not Available	
o,p'-DDD	29		
cis-Nonachlor	18		

Table 10 compares the measured organochlorine pesticide and PCB values at fish kill sites with minimum benchmark screening criteria. None of the organochlorine pesticide levels measured at fish kill sites exceeded published benchmark screening criteria. Measured values ranged from 6.36 times to 1.57 x 10^6 times below published screening criteria. Measured concentrations of chlorpyrifos were the closest to screening criteria (at 6.36 times below screening criteria values).

Table 10. Comparison of Measured Organochlorine Pesticide and PCB Concentrations to Benchmark Screening Criteria.

Compound	Maximum Measured Value (pg/L)	Minimum Benchmark Screening Level (ug/L)	Ratio of Screening Level to Measured Value
Chlorpyrifos	550	0.0035	6.36E+00
Hexachlorobenzene (HCB)	35	0.0003	8.57E+00
Dieldrin	56	0.0019	3.39E+01
Lindane	240	0.01	4.17E+01
Endrin	36	0.002	5.56E+01
p,p'-DDD	79	0.0064	8.10E+01
Heptachlor Epoxide	42	0.0038	9.05E+01
Endosulfan-II	510	0.056	1.10E+02
Endosulfan Sulfate	310	0.056	1.81E+02
o,p'-DDD	29	0.0064	2.21E+02
p,p'-Methoxychlor	56	0.019	3.39E+02
Heptachlor	1.2	0.0038	3.17E+03
beta-Benzenehexachloride (b-BHC)	110	0.495	4.50E+03
Trifluralin	19	0.2	1.05E+04
delta-Benzenehexachloride (d-BHC)	75	2.2	2.93E+04
p,p'-DDE	64	10.5	1.64E+05
alpha-Benzenehexachloride (a-BHC)	47	74	1.57E+06
Pentachloroanisole (PCA)	59	No Benchmark Screening Data Available	
Dacthal	29		
Oxychlordane	7.1		
trans-Chlordane	37		
trans-Nonachlor	38		
cis-Chlordane	54		
cis-Nonachlor	18		
o,p'-DDT	21		
cis-Permethrin	120		
trans-Permethrin	140		
Total PCBs	1600		

Table 11 compares the measured organochlorine pesticide and PCB values at fish kill sites with 90th percentile statewide SMPD data. Nine organochlorine pesticides and PCBs exceeded the statewide 90th percentile. Chlorpyrifos values were the highest compared to statewide percentiles, exceeding the statewide 90th percentile by nearly 18 times. Maximum values of delta-benzenehexachloride (d-BHC) and p,p'-methoxychlor were also greater than 10 times the statewide 90th percentile, and total PCBs were 5 times the statewide 90th percentile. All other compounds were less than twice the statewide 90th percentile.

Table 11. Comparison of Measured Organochlorine Pesticide and PCB Concentrations to Statewide 90th Percentile.

Compound	Maximum Measured Value (pg/L)	90th Percentile of Statewide Data (pg/L)	Ratio of Statewide 90th %tile Level to Measured Value
Chlorpyrifos	550	31	0.0563636
delta-Benzenehexachloride (d-BHC)	75	5	0.0666667
p,p'-Methoxychlor	56	5	0.0892857
Total PCBs	1600	321	0.200625
beta-Benzenehexachloride (b-BHC)	110	73	0.6636364
Lindane	240	169	0.7041667
Dacthal	29	26	0.8965517
p,p'-DDD	79	71	0.8987342
Hexachlorobenzene (HCB)	35	32	0.9142857
p,p'-DDE	64	59	0.921875
trans-Nonachlor	38	53	1.3947368
cis-Chlordane	54	85	1.5740741
trans-Chlordane	37	61	1.6486486
Heptachlor Epoxide	42	81	1.9285714
o,p'-DDD	29	59	2.0344828
Dieldrin	56	118	2.1071429
Endrin	36	85	2.3611111
alpha-Benzenehexachloride (a-BHC)	47	111	2.3617021
Heptachlor	1.2	3	2.5
o,p'-DDT	21	87	4.1428571
Pentachloroanisole (PCA)	59	275	4.6610169
Trifluralin	19	122	6.4210526
Oxychlordane	7.1	76	10.704225
cis-Nonachlor	18	No Statewide Probabilistic Data Available	
Endosulfan-II	510		
Endosulfan Sulfate	310		
cis-Permethrin	120		
trans-Permethrin	140		

In summary, several organochlorine pesticides and PCBs at fish kill locations were elevated, based on statewide percentiles. This is not surprising, considering that the Shenandoah River basin is one of the most agricultural areas of the state. Despite the elevated concentrations based on statewide percentiles, none of the organochlorine pesticides or PCBs exceeded effect levels or benchmark screening criteria. Based on this comparison, passive sampler data provided no evidence that any organochlorine pesticide or PCB is a primary cause of fish kills in the Shenandoah and James River basins.

In general, the organochlorine pesticides were closer to effect levels and benchmark screening criteria than PAHs measured in passive samplers. Chlorpyrifos was consistently the compound closest to effect levels and benchmark screening criteria. Chlorpyrifos exceeded control values at 7 of the 10 fish kill sites, was nearly 18 times the statewide 90th percentile, was within an order of magnitude of benchmark screening criteria, was within 2 orders of magnitude of Virginia water quality standards, and was within 3 orders of magnitude of lethal effect levels for fish.

1.4.3. Agricultural Pesticides

A total of 35 agricultural pesticides were analyzed from POCIS extracts. This group of pesticides consists of more polar compounds than the group of organochlorine pesticides just discussed. This group of agricultural pesticides was sequestered by POCIS, while the organochlorine pesticides were sampled by SPMDs. Of the 35 agricultural pesticides analyzed in POCIS extracts, 24 were not detected at any site. The remaining 11 compounds exceeded control values at one or more fish kill sites (Table 12). Four compounds (atrazine, simazine, metolachlor, and prometon) exceeded control values at 8 or more fish kill sites. Measured values at individual sites exceeded control values by 2 to 3611 times. The highest measured values were for atrazine, a popular herbicide commonly used in corn production. Atrazine was not detected at the 2 control sites (<0.18ng/L), but was as high as 650 ng/L at NF-Mt. Jackson. Many of the pesticide levels were highest at NF-Mt. Jackson or NF-Woodstock, where two sequential passive samplers were deployed. At these stations, pesticide levels were higher in the second deployment (from end of April to beginning of June) than the first (mid March to end of April). This is likely related to the timing of agricultural pesticide usage in the study area.

Table 12. Comparison of Agricultural Pesticide Levels at Fish Kill Sites with Control Sites.

Compound	# Sites Exceeding Control Values	Maximum Measured Value (ng/L)	Location of Maximum Value	Ratio of Maximum Concentration to Control Value
Atrazine	8	650	NF-Mt. Jackson	3611.1
Simazine	8	24	NF-Mt. Jackson	126.3
Metolachlor	9	11	NF-Woodstock	110.0
trans-Permethrin	3	30 ng/POCIS	North R.	62.5
cis-Permethrin	3	86 ng/POCIS	North R.	57.3
Prometon	8	4.2	NF-Mt. Jackson, NF-Woodstock	46.7
Atraton	1	1.8	North R.	22.5
Carbaryl	2	40 ng/POCIS	NF-Mt. Jackson	20.0
Simetryn	1	1.8	North R.	16.4
Desethylatrazine	5	37	Linville C.	4.3
Pendimethalin	1	0.32	Cowpasture R.	2.0
EPTC	Not Detected At Any Site			
Desisopropylatrazine				
Trifluralin				
Dimethoate				
Propazine				
Terbuthylazine				
Fonofos				
Cyromazine				
Diazinon				
Metribuzin				
Acetochlor				
Methyl Parathion				
Alachlor				
Ametryn				
Prometryn				
Terbutryn				
Malathion				
Chlorpyrifos				
Dacthal				
Fipronil				
Ethopabate				
Endosulfan I				
Tetrachlorvinphos				
Endosulfan II				

Of the 11 pesticides detected, 3 were reported only as concentrations in the extract and not water column concentrations (trans-permethrin, cis-permethrin, and carbaryl). Because these compounds were not reported as water column concentrations, they could not be compared to effect level data or benchmark screening criteria. Virginia water quality standards and statewide

passive sampler data were not available for any of the pesticide compounds, so comparisons against those thresholds were not possible.

Table 13 compares the measured agricultural pesticide values at fish kill sites with minimum effect levels. None of the compounds measured at fish kill sites exceeded published lethal effect levels. Measured values were 4 to 7 orders of magnitude below relevant effect levels. Atrazine values were the highest (650 ng/L) and were the closest to published effect levels (1.03×10^4). Effect level data were not available for atraton and desethylatrazine, a breakdown product of atrazine.

Table 13. Comparison of Measured Agricultural Pesticide Concentrations to Literature Cited Effect Levels.

Compound	Maximum Measured Value (ng/L)	Minimum Lethal Effect Level (ug/L)	Ratio of Effect Level to Measured Value
Atrazine	650	6700	1.03E+04
Metolachlor	11	3200	2.91E+05
Simazine	24	10000	4.17E+05
Pendimethalin	0.32	199	6.22E+05
Prometon	4.2	15500	3.69E+06
Simetryn	1.8	25000	1.39E+07
Atraton	1.8	Effect Level Data Not Available	
Desethylatrazine	37		
trans-Permethrin	30 ng/POCIS	Water Column Concentrations Not Estimated	
Carbaryl	40 ng/POCIS		
cis-Permethrin	86 ng/POCIS		

Table 14 compares measured agricultural pesticide levels at fish kill sites with benchmark screening criteria. None of the compounds exceeded benchmark screening criteria. Measured values ranged from almost 3 times below screening levels to 2 orders of magnitude below screening levels. Atrazine was the closest to benchmark screening critieria at just 2.77 times lower. Benchmark screening criteria were not available for 5 of the agricultural pesticide compounds.

Table 14. Comparison of Measured Agricultural Pesticide Concentrations to Benchmark Screening Criteria.

Compound	Maximum Measured Value (ng/L)	Minimum Benchmark Screening Level (ug/L)	Ratio of Screening Level to Measured Value
Atrazine	650	1.8	2.77E+00
Metolachlor	11	7.8	7.09E+02
Simazine	24	10	4.17E+02
Pendimethalin	0.32	No Benchmark Screening Data Available	
Prometon	4.2		
Simetryn	1.8		
Atraton	1.8		
Desethylatrazine	37		
trans-Permethrin	30 ng/POCIS	Water Column Concentrations Not Estimated	
Carbaryl	40 ng/POCIS		
cis-Permethrin	86 ng/POCIS		

In summary, no agricultural pesticide exceeded published lethal effect levels or benchmark screening criteria. Several pesticides exceeded control values at a majority of fish kill stations, but none exceeded control values at all fish kill stations. Of the agricultural pesticides, atrazine was measured at the highest concentration, it exceeded control values by the largest amount (3611 times), it was the closest to lethal effect levels (1.03×10^4 times lower), and it was the closest to benchmark screening criteria (2.77 times lower).

1.4.4. Waste-Indicator Chemicals

A total of 61 waste-indicator chemicals were analyzed from passive samplers. These chemicals were reported as concentrations in passive sampler extracts, and were not translated to water column concentrations. For this reason, waste-indicator chemicals cannot be compared against water quality standards, effect level data, or benchmark screening criteria. Statewide probabilistic monitoring data are also not available for these compounds. The results analysis for this group of compounds is limited to comparing fish kill stations to control stations.

Table 15 compares measured waste-indicator chemical levels at fish kill sites to control sites. Of the 61 waste-indicator chemicals, 42 were not detected at any station. Another 2 waste-indicator

chemicals were less than control values at all fish kill stations. The remaining 17 chemicals exceeded control values at 1 or more locations. Measured levels of waste-indicator chemicals at fish kill stations ranged from 1 to 250 times control values. No compound exceeded control values at more than 5 fish kill stations. Atrazine was the highest measured waste-indicator chemical measured in passive sampler extracts and also exceeded control values by the largest amount (250 times).

Table 15. Comparison of Waste-Indicator Chemical Levels at Fish Kill Sites with Control Sites.

Compound	# Sites Exceeding Control Values	Maximum Measured Value (ng/POCIS)	Location of Maximum Value	Ratio of Maximum Concentration to Control Value
Atrazine	5	5000	Linville C.	250.0
Indole	5	1200	South R.	60.0
para-Cresol	2	120	NF-Woodstock	6.0
Cholesterol	4	3500	SF-Whitehouse	4.9
N,N-diethyltoluamide (DEET)	2	80	NF-Woodstock	4.0
Caffeine	2	70	NF-Woodstock	3.5
Benzo[a]pyrene	3	40	SF-Whitehouse	2.0
Prometon	2	30	Linville C.	1.5
Diethylhexylphthalate (DEHP)	5	890	Linville C.	1.5
Diethyl phthalate	3	80	Shen-Berryville, Linville C.	1.3
Galaxolide (HHCB)	1	260	South R.	1.3
Isophorone	2	20	NF-Strasburg, Linville C.	1.0
Tonalide (AHTN)	2	20	North, South R.	1.0
Benzophenone	1	20	NF-Cootes Store	1.0
Fluoranthene	1	20	SF-Whitehouse	1.0
Pyrene	1	20	SF-Whitehouse	1.0
Tri(dichloroisopropyl) phosphate	1	20	NF-Woodstock	1.0
Tri(2-chloroethyl) phosphate	0	Less Than Control Value At All Sites		
Carbazole	0			
Tetrachloroethylene	Not Detected At Any Site			
Bromoform				
Isopropylbenzene (cumene)				
Phenol				
1,4-Dichlorobenzene				
d-Limonene				
Acetophenone				
Camphor				
Menthol				
Naphthalene				

Methyl salicylate
Dichlorvos
Isoquinoline
2-Methyl naphthalene
1-Methyl naphthalene
2,6-Dimethylnaphthalene
Cashmeran (DPMI)
p-tert-Octylphenol
Tributyl phosphate
Ethyl citrate
Cotinine
Celestolide (ADBI)
Phantolide (AHMI)
4-Octylphenol
N-butyl benzenesulfonamide
Pentachlorophenol
Phenanthrene
Anthracene
Diazinon
Musk Ambrette
Traseolide (ATII)
Musk Xylene
Carbaryl
Metalaxyl
Bromacil
Anthraquinone
Musk Ketone
Chlorpyrifos
Triclosan
Bisphenol A
Tri(butoxyethyl) phosphate
Triphenyl phosphate

1.4.5. Pharmaceuticals

A total of 30 pharmaceuticals were analyzed from passive samplers. These compounds were reported as concentrations in passive sampler extracts, and were not translated to water column concentrations. For this reason, pharmaceuticals cannot be compared against water quality standards, effect level data, or benchmark screening criteria. Statewide probabilistic monitoring data are also not available for these compounds. The results analysis for this group of compounds is limited to comparing fish kill stations to control stations.

Table 16. Comparison of Pharmaceutical Levels at Fish Kill Sites with Control Sites.

Compound	# Sites Exceeding Control Values	Maximum Measured Value (ng/POCIS)	Location of Maximum Value	Ratio of Maximum Concentration to Control Value
Caffeine	5	140	NF-Woodstock	24.6
Codeine	2	7	NF-Strasburg	2.4
Trimethoprim	3	100	North R.	1.9
1,7-dimethylxanthine	0	Less Than Control Value At All Sites		
Carbamazepine	0			
Cotinine	0			
Sulfamethoxazole	0			
Venlafaxine	0			
Acetaminophen	Not Detected At Any Site			
Albuterol				
Azithromycin				
Cimetidine				
Dehydronifedipine				
Diltiazem				
Diphenhydramine				
Erythromycin				
Miconazole				
Ranitidine				
Thiabendazole				
Warfarin				
Bupropion				
Citalopram				
Duloxetine				
Fluoxetine				
Fluvoxamine				
Norfluoxetine				
Norsertraline				
Paroxetine				
Paroxetine Metabolite				
Sertraline				

Table 16 compares measured pharmaceutical levels at fish kill sites to control sites. Of the 30 pharmaceuticals, 22 were not detected at any station. Another 5 pharmaceuticals were less than control values at all fish kill stations. The remaining 3 pharmaceuticals (caffeine, codeine, and trimethoprim) exceeded control values at 1 or more locations. No pharmaceutical exceeded control values at more than 5 fish kill sites. Maximum values of those pharmaceuticals exceeded control values by 2 to 25 times.

1.4.6. Hormones

A total of 4 selected hormones were analyzed from passive samplers. None of the hormones exceeded control values at more than 3 fish kill sites (Table 17). Levels of all 4 hormones were highest at SF-Whitehouse, where levels exceeded control values by 2.9 to 8.9 times. Values of the synthetic hormone, 17a-ethynylestradiol, were the highest (8.1 ng/L) and exceeded control values by the greatest amount (8.9 times).

Table 17. Comparison of Selected Hormone Levels at Fish Kill Sites with Control Sites.

Compound	# Sites Exceeding Control Values	Maximum Measured Value (ng/L)	Location of Maximum Value	Ratio of Maximum Concentration to Control Value
17a-Ethynylestradiol	3	8.1	SF-Whitehouse	8.9
Estriol	2	3.4	SF-Whitehouse	6.1
17b-Estradiol	3	2.3	SF-Whitehouse	5.0
Estrone	1	1.6	SF-Whitehouse	2.9

Table 18 compares the measured hormone values at fish kill sites with minimum effect levels. None of the hormones measured at fish kill sites exceeded published lethal effect levels. Maximum measured values of 17a-Ethynylestradiol were 12.3 times below lethal effect levels, and maximum measured values of 17b-Estradiol were 500 times below lethal effect levels. Effect level data were not available for estrone or estriol. No benchmark screening criteria, statewide probabilistic, or water quality standards were available for these hormones for comparison. It should be noted that while hormone levels in passive samplers are below reported lethal effect levels, they are above levels reported as producing effects on sexual development (Lange *et al.*, 2008). These data support the finding of a high incidence of intersex in fish from the Potomac River drainage (Blazer *et al.*, 2007).

Table 18. Comparison of Measured Hormone Concentrations to Literature Cited Effect Levels.

Compound	# Sites Exceeding Control Values	Maximum Measured Value (ng/L)	Location of Maximum Value	Minimum Lethal Effect Level (ug/L)	Ratio of Effect Level to Measured Value
17a-Ethynylestradiol	3	8.1	SF-Whitehouse	0.1	1.23E+01
17b-Estradiol	3	2.3	SF-Whitehouse	1.15	5.00E+02
Estrone	1	1.6	SF-Whitehouse	Effect Level Data Not Available	
Estriol	2	3.4	SF-Whitehouse		

1.4.7. Yeast Estrogen Screen

A yeast estrogen screen was used to assess the combined estrogenicity of compounds sequestered by the passive samplers. The results of this analysis were reported as concentrations in passive sampler extracts, and were not translated to water column concentrations. For this reason, the yeast estrogen screen results cannot be compared against water quality standards, effect level data, or benchmark screening criteria. Statewide probabilistic monitoring data are also not available for this parameter, so the results analysis is limited to comparing fish kill stations to control stations. The estrogenicity of samples, measured as estradiol equivalent factors, ranged from 2.3 to 79 E2ng/POCIS. The lowest values were observed at the Cowpasture R. and Shen-Berryville sites, and the highest value was observed at NF-Woodstock. Only 4 sites exceeded control values, and the NF-Woodstock site exceeded control values by 10 times.

1.5. DISCUSSION AND CONCLUSIONS

Passive sampler data collected from the Shenandoah and James River basins in 2007 produced no evidence that fish kills are a direct result of chemical contamination in the water column. Passive sampler extracts were analyzed for nearly 200 constituents, and 102 compounds were detected at one or more sites, however, no compound at any site exceeded minimum lethal effect thresholds or published benchmark screening criteria. All compounds that were expressed as water column concentrations were orders of magnitude lower than minimum published lethal effect levels. 17a-ethynylestradiol was the closest to minimum lethal effect levels at 12.3 times lower. The next closest compound was chlorpyrifos at 527 times lower. The only compounds within an order of magnitude of minimum published benchmark screening criteria were atrazine (at 2.77 times lower), chlorpyrifos (at 6.36 times lower), and hexachlorobenzene (at 8.57 times lower).

1.5.1. Comparison with Control Sites

The fish kills experienced in the Shenandoah and James River basins over the past few years have displayed a distinct set of characteristics, suggesting a common cause. If this is the case, then the causal factor should be present at most, if not all, of the fish kill locations. Likewise, if a water quality parameter is directly or indirectly involved in producing the fish kills, then that

parameter should be found at higher concentrations at fish kill sites than control sites that did not experience fish kills. Based on this premise, any causal agent would be expected to exceed control concentrations at most, if not all, fish kill sites. Of the 199 compounds analyzed, only 6 exceeded control site values at a majority of (i.e., more than 5) fish kill sites. All 6 were agricultural pesticides. Metolachlor exceeded control values at 9 of 10 fish kill sites. Atrazine, simazine, and prometon exceeded control values at 8 fish kill sites. Chlorpyrifos and p,p'-methoxychlor exceeded control values at 7 and 6 fish kill sites, respectively.

Based on comparisons with control sites, only agricultural pesticides fit the spatial pattern of fish kills. Other parameter groups do not fit the same spatial pattern. This finding is expected, since fish kill watersheds have a much larger proportion of agricultural lands than control site watersheds. The spatial fit of agricultural pesticides and fish kills, however, should not be mistaken for a causal relationship. There are some obvious discrepancies with the spatial pattern, such as the Cowpasture River results (see Section 1.5.3), and comparison of measured values with published effect levels also does not support a causal relationship (see Section 1.5.2).

1.5.2. Comparison with Effect Levels

Where possible, measured contaminant levels were compared to Virginia water quality criteria, statewide probabilistic monitoring results, minimum reported lethal effect levels (in EPA's ECOTOX database), and minimum published benchmark screening criteria. Comparison with Virginia water quality criteria was not very informative, since water quality criteria were only available for 5 parameters. Statewide probabilistic monitoring results were available for the PAHs, organochlorine pesticides, and PCBs. A total of 4 PAHs, 9 organochlorine pesticides, and total PCBs exceeded statewide 90^{th} percentiles at 1 or more fish kill locations. This is not surprising, since some fish kill watersheds contain more urban and agricultural influences than is typical across the state. Similarly to comparisons with control sites, comparisons to statewide probabilistic monitoring results do not indicate a causal relationship. To suggest a causal relationship, it is necessary to compare measured concentrations with levels demonstrated to cause a similar effect in laboratory or field studies.

Comparison with effect levels revealed that no compound at any site exceeded minimum published lethal effect levels. All compounds were found at concentrations orders of magnitude

lower than minimum published lethal effect levels. PAHs were more than 4000 times below minimum lethal effect levels, organochlorine pesticides were more than 500 times below minimum lethal effect levels, agricultural pesticides were more than 10,000 times below minimum lethal effect levels, and hormones were more than 12 times below minimum lethal effect levels.

Passive sampler results were also compared against minimum published benchmark screening criteria. These benchmark screening criteria are typically much lower than lethal effect levels for fish, because they consider a wide variety of aquatic species (fish, invertebrates, and algae), they consider sublethal and bioaccumulative effects, and they often include margins of safety or risk factors. Even with the conservative nature of benchmark screening criteria, no compounds at any site exceeded these criteria. The only compounds within an order of magnitude of minimum published benchmark screening criteria were atrazine (at 2.77 times lower), chlorpyrifos (at 6.36 times lower), and hexachlorobenzene (at 8.57 times lower). All other compounds at fish kill sites were more than an order of magnitude below benchmark screening criteria.

1.5.3. Results from the Cowpasture River

The passive sampler results from the Cowpasture River provide additional evidence that recurring fish kills are not likely due to chemical contamination or water quality parameters examined in this study. During the period of passive sampler deployment, the Cowpasture River experienced some of the worst fish kills observed in the Shenandoah or James River basins in 2007. Contaminant concentrations in passive samplers from the Cowpasture River, however, were among the lowest. Of the 199 parameters measured in passive sampler extracts, only 21 were even detected in the Cowpasture River. This included 8 PAHs, 8 organochlorine pesticides and PCBs, 1 agricultural pesticide, 2 waste-indicator compounds, 1 pharmaceutical, and the yeast estrogen assay. Of the 21 compounds detected in the Cowpasture River, only 3 exceeded values from the control sites (4-methylbiphenyl, pendimethalin, and caffeine), and these are not likely contributors to fish kills. Pendimethalin concentrations in the Cowpasture River were more than 5 orders of magnitude below minimum effect levels, and caffeine is not toxic at environmentally relevant concentrations. Passive samplers from the Cowpasture River accumulated only 10 ng/POCIS of caffeine, and the lowest effect level published in EPA's ECOTOX database for

caffeine was a Lowest Observable Effect Concentration (LOEC) of 20,000 ug/L (DeYoung *et al.*, 1996). Effect level data were not available for 4-methylbiphenyl, but, the 500 pg/L concentration measured in the Cowpasture River is more than 3 orders of magnitude lower than the lowest lethal effect level published for any of the PAHs. In addition, the toxicity of PAHs is roughly additive (Erickson, *et al*., 1999), and the Cowpasture River showed lower total PAH concentrations than either of the two control sites. Passive sampler results from the Cowpasture River suggest that fish kills are not caused by chemical contaminants in the water column, unless the culprit is some constituent not analyzed in this study and not correlated with the groups of contaminants that were measured.

1.5.4. Unanalyzed Compounds and Compound Mixtures

The passive sampler and analytical techniques used in this study are the state-of-the-art for environmental sampling of low-level contaminants. Despite this fact, only 199 individual compounds could be analyzed. This is a small fraction of the more than 83,000 chemicals registered under the Toxic Substances Control Act (TSCA) Inventory (USEPA, 2008b). While many classes of compounds were analyzed, many more remain, including: metals, antibiotics, alkylphenol ethoxylates, polybrominated diphenyl ether flame retardants, dioxins, and furans. This study did not find any evidence that fish kills were the result of chemical contamination in the water column, however, this conclusion must be reserved for the 199 chemicals tested. This study does not rule out the possibility that other contaminants that were not tested could be responsible for Shenandoah and James River fish kills.

Another limitation of this study is that the effects of compound mixtures were not considered. Compound mixtures may produce toxic effects that are simply additive, or the compounds may act synergistically to produce increased toxicity, or antagonistically to reduce toxicity (Landis and Yu, 1999). Since all individual contaminants monitored were orders of magnitude below published effect thresholds, it is not likely that chemical mixture effects could account for the observed fish kills. Using a simple additive toxic units model, the additive toxicity of all compounds from this study exceeding control values was only 0.086 toxic units. This is still more than an order of magnitude below a toxic unit of greater than 1, which would be expected to produce toxic effects. This implies that even assuming additive toxicity across all contaminants (which is unrealistically conservative because the chemical classes monitored have

different toxic modes of action), the combined mixture would not be expected to produce toxic effects.

1.5.5. Sublethal Effects

One concern of the Fish Kill Task Force is that contaminants may not be directly causing fish kills, but may be producing sublethal effects that indirectly contribute to the kills. Chemical contamination that affects the immune system could make fish more susceptible to pathogens that then cause infection and ultimately death. Such immune system effects have been documented for numerous contaminants, however, effect levels for immune system responses are typically well above levels measured in passive samplers during this study.

Arkoosh and Collier (2002) demonstrated immune function impairment in salmon from contaminated urban estuaries in Puget Sound with high PAH and PCB levels. In laboratory experiments, Arkoosh *et al.* (1994) determined that immune system effects were observed at PAH concentrations that were 1% of lethal doses and PCB concentrations that were 20% of lethal doses. Karrow *et al.* (1999) documented immune system effects in rainbow trout exposed to creosote at levels above 612 ng/L total PAH. The levels of immune system response to PAHs and PCBs found in the above studies are well above levels measured in passive samplers from the Shenandoah and James River basins.

Blakley *et al.* (1999) and Galloway and Handy (2003) published reviews of research on the immunotoxicity of pesticides. Blakley *et al.* (1999) cited immune system effects from lindane in rainbow trout, tilapia, and carp at relatively high oral doses (1-1000 ppm). The insecticide mirex did not produce significant immune system responses in rainbow trout exposed for 12 months at 0.5 to 50 ppm. Galloway and Handy (2003) cited studies of malathion and diazinon showing immune system effects in *Oryzias latipes* at 0.2-0.8 mg/L malathion and in *Lepomis macrochirus* at 15-75 ug/L diazinon. Lastly, Fatima *et al.* (2007) demonstrated immune system effects in goldfish exposed to an herbicide mixture of atrazine, simazine, diuron, and isoproturon at 50 ug/L. All of these demonstrated immune system effect levels remain orders of magnitude above levels measured in passive samplers.

While this study showed no evidence that contaminant levels are adequate to induce immune suppression in fish kill rivers, the availability of immunosuppression literature with which to compare contaminant levels is limited. Additional research is necessary to investigate the potential role of immunosuppression in fish kill rivers. Ripley *et al.* (2008) have identified variations in immune response among fish kill rivers, yet comparisons of immune response between fish kill and control rivers have not been investigated. Additional research is also needed to link chemical contaminant levels in the Shenandoah and James River basins to possible immune suppression effects.

1.5.6. Recommendations

The objective of passive sampler analysis was to screen fish kill sites for a large number of organic chemicals. In this respect, the study was successful, and this broad screening has provided a baseline for characterizing organic contamination at fish kill locations. The hope was also that passive sampler data might identify a single or small subset of contaminants that could potentially be responsible for causing the observed fish kill symptoms. This finding could direct future investigations and speed the progress of a solution for Shenandoah and James River basin fish kills. Passive sampler data, however, did not identify any contaminants at levels that would suggest a cause of observed fish kills. Nonetheless, the analysis and conclusions of this report suggest several recommendations regarding the direction and scope of fish kill investigations. These recommendations are as follows:

- Fish kill investigators and Fish Kill Taskforce members should continue to view contaminant data within the context of published effect levels for those contaminants. The passive sampler methodology allows monitoring of contaminants at very low levels (e.g., pg/L), so the mere detection of a contaminant should not be confused with biological significance. Many persistent organic chemicals are ubiquitous in the environment, so the number of contaminants detected will continue to increase as analytical procedures improve and detection limits decrease.

- Water quality studies should continue to include and should seek to identify new control or reference sites unaffected by fish kills. The comparison of water quality information between fish kill affected and unaffected sites is key to identifying potential causes. This

study utilized 3 control sites, however, 1 of those sites (the Cowpasture River) experienced fish kills in 2007. This possibility should be recognized in selecting the number and location of future control sites.

- Future passive sampler studies should use robust quality control measures including field blanks at each location. In this study, passive samplers deployed by the Friends included field blanks, but samplers deployed by VADEQ did not include field blanks. This resulted in some discrepancies among the detection and quantification levels achieved at the various sites.

- Additional literature searches should be conducted to establish effect levels for immune suppression endpoints. This study focused primarily on lethal effect levels, since lethality has been the ultimate observed symptom at fish kill locations. Several published reviews of immune suppression literature were considered in this study, but an exhaustive literature search was not conducted for each contaminant.

- Future water quality studies should have defined objectives that build upon the findings of existing fish kill studies. The passive sampler study, storm event monitoring study, continuous ammonia monitoring study, and routine VADEQ water quality monitoring have provided a robust dataset for characterizing water quality conditions in fish kill areas. Future water quality studies should either attempt to expand the dataset by examining additional contaminants that have not yet been targeted or focus on particular constituents as directed by fish health, pathology, or microbiological findings.

- Future investigations should include controlled exposure studies that begin to experimentally link environmentally-relevant contaminant concentrations or pathogens with effects observed in the fish kill rivers.

1.6. REFERENCES

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Appendix A:

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Appendix B:

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